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Research article

Markers of inflammation, endothelial activation and autoimmunity in adolescent female gymnasts

Eyad Alshammari [1], Shahida Shafi [1], Jaana Nurmi-Lawton [1], Dayangku Fatiha Pengiran Burut [1], Susan Lanham-New [1] and Gordon Ferns [1,2] ✉
[1] Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK
[2] Guy Hilton Research Centre, Institute of Science & Technology in Medicine, Stoke on Trent, Staffordshire, UK

Abstract
High levels of physical activity have been linked to benefits in cardiovascular and bone health by affecting, in part, changes in proinflammatory profile. Therefore, we have aimed to assess the effects of intensive training on markers of inflammation, endothelial activation and auto-immunity in the absence of the potential confounding effects of incident atherosclerosis. The subjects comprised 25 competitive gymnasts and 19 healthy sedentary adolescent females, aged 8-17 years. Serum soluble intercellular adhesion molecule 1 (sICAM-1), high sensitivity C-reactive protein (hsCRP), heat shock protein 27 (Hsp27) and Hsp27 antibody titres were measured by ELISAs in a sample of adolescent girls who were either physically active (competitive gymnasts) or sedentary. The association between age, body mass index (BMI), dietary intake, serum hsCRP, sICAM-1 and Hsp27 antigen and antibody titres were determined. The mean serum sICAM-1 concentrations were significantly higher in the gymnasts compared to the sedentary females (0.29 ± 0.02 versus 0.23 ± 0.01 $mg{\cdot}L^{-1}$, $p < 0.01$). In contrast serum hsCRP concentrations were substantially lower in the gymnasts compared to the sedentary adolescent females (0.49 ± 0.03 versus 1.38 ± 0.19 $mg{\cdot}L^{-1}$, $p < 0.001$). Differences remained significant after adjustment for anthropometric factors. We also found that serum Hsp27 antigen concentrations were determined by dietary saturated fat intake ($p < 0.001$), and antibody titres to Hsp27 were determined by dietary PUFA ($p < 0.001$) after adjustment for BMI. Our findings show that young female gymnasts have an altered profile of inflammatory markers and endothelial activation compared to their less physically active peers.

Key words: Physical activity, dietary intakes, hsCRP, sICAM-1, Hsp27, antibodies.

Introduction

Physical activity is associated with reduced serum concentration of inflammatory markers. Previous studies have shown that the type and the degree of exercises increases oxidative stress, which, in turn causes the generation of reactive oxygen species (ROS), that influences inflammatory markers including C-reactive protein (CRP) (Kasapis and Thompson, 2005; Majka et al., 2009) and genetic factors (Shen and Ordovas, 2009). Athletes have also been reported to have better endothelial function than sedentary controls (Franzoni et al., 2004), although, prolonged, brisk exercise is also reported to transiently raise markers of endothelial activation, such as ICAM-1 and E-selectin (Bartzeliotou et al., 2007). In the light of these studies we have hypothesed that intensive training alters the markers of inflammation, endothelial functions and autoimmunity in adolescent females.

Reactive oxygen species (ROS) are generated during exercise from several sources including leakage from the electron transport chain. These ROS may cause cellular damage that may subsequently lead to lipid oxidation, protein denaturation or DNA damage (Droge, 2002). However, exercise is also reported to enhance the endogenous antioxidant defences (Rousseau et al., 2006). For example, repeated episodes of aerobic exercise has been reported to induce the expression of antioxidant enzymes (Ji, 2002), and several studies have shown a relationship between extent of physical activity and plasma antioxidant concentrations (Dekany et al., 2006). Furthermore Pialoux and his colleagues have reported that the intensity of exercise and exposure to hypoxia may have a cumulative effect on oxidative stress (Pialoux et al., 2006) and that though antioxidant defences are enhanced in elite athletes this does not allow them sufficient buffering to counterbalance the over-production of free radicals during exercise (Pialoux et al., 2009). The potential damaging effects of reactive oxygen species can be partially reversed by molecular chaperones, such as heat shock protein 27 (Hsp27) that may be involved in a process of protein renaturation (Ferns et al., 2006). However, during this process the chaperone molecules appear to be altered in a way that may render them antigenic (Wick et al., 2004).

The principle aim of this current study was to determine weather intensive exercise alters markers of inflammation, endothelial activation and autoimmunity by comparing adolescent female gymnasts, engaged in high physical activity with sedentary girls, matched for associations between age and associations between them. Using this young age group of subjects removed the potential confounding effects of existing vascular disease. The term intensive training/exercise refers to a physical activity related to the average number of hours per day of gymnastic training which incorporates impact exercise including evaluating jumping, tumbling and strength work. The specific objectives were to assess their serum levels of CRP, sICAM-1 and Hsp27 antigen and antibodies, and to determine their association with age, adiposity and dietary intakes of macro- and micro-nutrients.

Methods

Subjects were recruited as part of a three-year longitude-

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nal investigation of the effects of exercise on peak bone mass (PBM) development as previously reported (Nurmi-Lawton et al., 2004). Twenty-five female gymnasts, 8-17 years of age were originally recruited from five athletics clubs in the South of England. The gymnasts were eligible to join the study if they trained > 10 h/week and regularly took part in competitions (at club regional level). Nineteen female healthy normally active controls were randomly recruited through the database of local General Practices (GPs) in Surrey, South England. Controls were involved in normal activities (including walking to school and PE classes) for on average 5.6 ± 2.6 h/week (determined by a checklist), but not in sports requiring all year training at competition level. In addition, anthropometric, physical activity and dietary intake were estimated as previously described (Nurmi-Lawton et al., 2004). Anthropometric data were determined by a nutritionist. None of the subjects had evidence of acute infection, or inflammation at the time of recruitment, or at the time of blood sample collection. Each gymnast was matched to a non-active control, initially by age and then subsequently by pubertal age once Tanner staging had been completed and analysed (Nurmi-Lawton et al 2004). The baseline data were collected between the months of October and December of the year for both gymnasts and controls. We were not able to collect information on the timing of the menstruation phase cycle during the collection of blood samples.

Ethical approval was obtained from the South-West Surrey local research ethics committee, Guildford, Surrey, and from University of Surrey ethics committee.

Dietary analysis

The dietary intake of gymnasts and controls was recorded for 7 days at baseline using estimated food diaries, which have been shown to have an acceptable relative validity (Bingham et al., 1994). Instructions, including how to estimate portion size, were given both verbally and in writing to each subject, or their parents by a registered dietician. Gymnasts were asked to complete the diary on a non-competition week, during the athletic training season and controls on a non-holiday week, without changing their usual dietary habits. The diaries were analyzed using Diet5 for Windows computer package (Robert Gordon University, Aberdeen, UK), which is based on McCance and Widdowson Food Composition Tables (Mark and Paul, 1992). This allowed an estimation of dietary macronutrient (protein, carbohydrate and fat) and micronutrients (including fat and water soluble antioxidants)

Assessment of menarche

Pubertal status was assessed at each measurement occasion, using self-assessment of secondary, sexual characteristics. Staging of secondary, sex characteristics according to Tanner TM (Tanner, 1962) has provided a means of assessing sexual development, but requires the individual to undress and then be physically examined. It is very difficult to collect such personal data in non-medical settings. Self assessment of the level of sexual maturation by adolescents has been found to be both valid and reliable, and in excellent agreement with physical examination (Morris and Udry, 1980), enabling accurate assessment of an individual's own developmental stage according to standard photographs by Tanner (Marshall and Tanner, 1969). In the present study, the use of adolescent self-staging was deemed most appropriate.

Physical activity assessment

The hours of training, changes in training regimen of each gymnast, and the type and amount of exercise undertaken by controls, were assessed using a questionnaire. The Blair score was calculated as described previously (Blair et al., 1985), which gives an indication of the individual's level of physical activity: <33 = very inactive, 33 to <37 = inactive, 37 to <40 = moderately active, ≥40 = active.

Blood collection

Non-fasted blood samples were collected from both the controls and gymnasts. Due to the recruitment procedure required for the gymnasts, that is, via gymnastic clubs that only met late afternoon/early evening, blood samples after a 12 hour fast were not possible. Control subjects predominantly attended the University of Surrey Clinical Investigation Unit (CIU) in the afternoon/early evening. Collections of samples for the gymnast group were carried out before training and out of competition week. Collections of samples for the controls were carried out during the school holiday. In all cases, they were collected around the time that the dietary estimates were obtained. Blood samples were centrifuged at 3000g at room temperature to obtain serum for analysis of markers of inflammation, endothelial activation, autoimmunity, antioxidants and trace elements. Serum samples were frozen at -80 ^{0}C until they were assayed.

Determination of ICAM-1 and hsCRP by ELISAs

Serum soluble intercellular adhesion molecule 1 (sICAM-1) and high sensitivity C-reactive protein (hsCRP) concentrations were measured in duplicate in all subjects using enzyme-linked immunosorbent assay (ELISA) kit purchased from [Quantikine (R & D Systems Europe, Ltd. U.K)], or an in-house ELISA respectively. hsCRP in-house ELISA: The plate was coated with 50 µl/well polyclonal rabbit anti-human CRP (diluted 1 in 4000 dilution in 0.1 M carbonate/bicarbonate buffer pH 9.6) and incubated overnight at 4 ^{O}C. The first three wells were coated with buffer alone as blanks. The following day, the plate was washed with washing buffer three times. Then 200 µl of blocking buffer was added to each well, and the plate was incubated for 60 minutes at 37^{o}C. The plate was washed again as above. 50 µl of samples (1:10) and standards diluted in PBS-T-0.2% BSA were added to the wells and incubated for 60 minutes at 37 ^{o}C. The plate was then washed four times with PBS containing 0.05% Tween-20. Then 50 µl of peroxidase-conjugated rabbit anti-human CRP was added to the wells, and the plate was incubated as above. After the incubation, the plate was first washed three times with PBS-T, then once with PBS, followed by two times with 0.05M citrate phosphate buffer (pH. 5). The reaction was initiated by the addition of 50 µl of TMB substrate solution and then incubating the plate in the dark for 3 minutes. The reaction was stopped by adding 12.5 µl of 2M H_2SO_4, and the plate was then read by micro plate reader (Victor^3, multilabel,

Singapore) at 450 nm.

The precision data for intra-assay variation was 3.6-5% (CV%) for ICAM1, 4.4-8.3% (CV%) for hsCRP; the inter-assay variation was 6.8-4.4% (CV%) for ICAM-1 and 6-6.6% (CV%) for hsCRP.

Determination of serum anti-Hsp27 antibody titres and Hsp27 antigen by ELISAs

All buffers, goat serum and chemicals were purchase from Sigma-Aldrich (Poole, UK) unless stated otherwise. Anti-human Hsp27 IgG and IgM antibody titres were measured in serum using an optimized in-house ELISA. Briefly, as previously described (Shams et al., 2008), a 96 well microtitre plate (Nunc), was coated with recombinant human Hsp27 protein (Bioquote Ltd, UK; 50 µl/well; 2 µg/ml in 0.1 M carbonate buffer pH 9.6) and incubated overnight at 4 °C. The plates were washed (x 3) with phosphate buffered-saline containing 0.05 % Tween-20 (PBS-T; pH 7.4) and any remaining binding sites were blocked with 2 % goat serum (300 µl/well). After incubation (30 minutes at 37 ºC) and washing (x 3 with PBS-T), serum samples from patients and controls diluted 1:100 were added (100 µl/well) in triplicate to both antigen-coated (target-) and uncoated, antigen-free (control) wells and the plates incubated for an additional 30 minutes at RT. After further washes with PBS-T (x 4) and PBS (x 2), bound Hsp-27 antibodies were detected by addition of a goat anti-human IgG-γ-chain/IgM-µ-chain specific horse-radish peroxidase-conjugated (HRP)-antibody [1:500; 100 µl/well; (Sigma-Aldrich, Poole, UK)]; the plates were then incubated for 30 minutes at RT. Both serum and conjugated antibodies were diluted in 2% goat serum. The enzymatic reaction was initiated by the addition (100 µl/well) of 0.01% tetramethylbenzidine substrate buffer to the wells. The reaction was stopped by the addition of 2 M HCl (100 µl/well) and the absorbance read at 450 nm in a microtitre plate reader (Perkin Elmer, Victor3™). Each assay included positive controls from sera with known high antibody titres to Hsp27 and negative controls (no antigen i.e. no human recombinant Hsp27 or no serum) on the same plate. After correction for the non-specific background absorbance (subtracting the absorbance of un-coated wells from the antigen-coated wells for each sample) the results were expressed in optical density units (Shams et al., 2008).

Serum Hsp27 antigen concentration was determined also using an optimized in-house ELISA. A microtitre plate was coated with monoclonal Hsp27 antibody (100 µl/well; 2.5µg/ml in PBS; SPA-800, Stressgen Bioreagents) and incubated as above. After blocking with 4% goat serum for 90 minutes, serum samples (diluted 1:3) and recombinant Hsp27 standards (0.94, 1.875, 3.75, 7.5 and 15 ng/ml; SPP-715, Stressgen Bioreagents) were added into duplicate wells (50 µl/well). After further incubation and washes, rabbit anti-human Hsp27 polyclonal antibody (1:6000 dilution; SPA-803, Stressgen Bioreagents) was added. The bound antibody was detected with goat-anti-rabbit HRP-conjugated antibody (1:6000 dilution) followed by enzymatic substrate reaction as above. The sensitivity of the assay was 0.94 ng/ml and the inter-assay and intra-assay coefficients of variation were 3.7% and 5.8% respectively.

Statistical analysis

Statistical analysis was performed using SPSS (version15). Data are expressed as mean ± SEM unless otherwise stated. Normality of the distribution of variables was confirmed using the Kolmogorov-Smirnov (K-S) test, and parametric or non-parametric tests were applied accordingly. Comparisons of anthropometric and other data at baseline between gymnasts and controls were performed using independent t-test or Mann-Whitney tests. A $p \leq 0.05$ was considered to be statistically significant. We initially used univariate model to investigate the association between hsCRP, sICAM-1, Hsp27 antigen and antibody titres and physical activity and adiposity. Stepwise multiple regression models were used to assess which of the confounding variable could influence hsCRP and ICAM-1.

Results

Comparison between anthropometric and demographic characteristic, dietary and energy intakes

The physical activity (PA) and anthropometric data height, body mass index (BMI), weight (WT), skin fold fat, mid arm circumstance and age of study subjects are shown in Table 1. As may have been expected the gymnasts showed 4 fold higher physical activity compared to controls. Compared with healthy adolescent females, the gymnasts were also shorter, with a lower BMI, mean weight, and % body fat as previously reported (Laing et al., 2002). These differences were statistically significant.

Energy and dietary intake of fat and trace elements determined by food diary at baseline are shown in Table 1. The gymnasts consumed significantly less total fat, particularly saturated fat and cholesterol, compared to the controls based on average daily intake baseline record ($p < 0.001$). They also had a significantly lower intake of selenium ($p < 0.001$). There were no significant differences in energy or dietary antioxidant (vitamins A, C and E) intake between the two groups.

Biochemical data

Serum sICAM was significantly higher in the gymnasts compared to the sedentary girls (0.29 ± 0.015 versus 0.23 ± 0.010 mg/L, mean ± SEM $p < 0.01$) whilst serum hsCRP concentrations were significantly lower (0.49 ± 0.03 versus 1.38 ± 0.19 mg/L, mean ± SEM $p < 0.001$) (Table 2). These differences remained significant after adjustment for body weight and height (ANCOVA, $p < 0.01$). No significant differences were observed in Hsp27 antigen and antibodies (IgG and IgM) concentrations.

Correlation between demographic data, dietary nutrients and markers of cardiovascular health

The association between anthropometric and demographic measurements, dietary nutrients and serum biochemical measures of inflammation (hsCRP), endothelial activation (ICAM-1), and response to cell stress (Hsp27 antigen and Hsp27 antibodies) were assessed using Pearson product moment correlation coefficients (Table 3). The results showed a significant, negative association between levels

Table 1. Comparison of demographic and anthropometric characteristics and dietary intakes derived from 7 day food diaries in gymnasts and controls. Values are expressed as mean (±SEM).

Variable	Controls (n=19)	Gymnasts (n=25)
Age (y)	12.00 (.46)	13.00 (.70)
PA (mins/ week)	304.00 (31.21)	1308.60 (38.06) ***
Height (m)	1.50 (.03)	1.40 (.02)
Weight (Kg)	42.60 (3.10)	34.00 (1.44) **
BMI (Kg/m^2)	19.20 (.77)	17.00 (.34) **
SFF (%)	23.50 (.77)	18.00 (.51) ***
MAC (cm)	23.60 (.73)	21.90 (.37) *
Macronutrients		
Energy (Kcal)	1822.5 (66.3)	1740.50 (79.77)
CHO(g)	246.70 (12.27)	237.00 (11.14)
Fat (g)	74.70 (2.94)	69.50 (3.99) ***
Protein (g)	56.70 (2.42)	56.40 (2.26)
SFA (g)	28.80 (1.11)	25.70 (1.61) ***
MUFA (g)	23.80 (1.07)	22.90 (1.32)
PUFA (g)	11.50 (.65)	12.40 (.85)
Chol (mg)	170.70 (13.48)	163.60 (10.55) ***
Micronutrients		
Se (mg)	36.10 (1.36)	34.00 (2.76) ***
Zinc (mg)	6.20 (.27)	5.90 (.30)
Vitamin A	635.30 (87.13)	727.70 (88.22)
Vit C (mg)	85.80 (11.85)	86.50 (14.23)
Vit E (mg)	4.90 (.67)	5.00 (.54)

Significance of difference compared to the controls; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. PA, physical activity; BMI, body mass index; SFF, skin fold fat; MAC, mid arm circumference; CHO, carbohydrates; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Chol, cholesterol; Se, selenium; Zn, zinc; Vit, vitamins.

of dietary factors (fat, SFA, and MUFAs) and ICAM-1 in gymnasts ($p < 0.05$). The negative association observed in this study between dietary factors and ICAM-1 concentrations has previously been reported for healthy subjects (Demerath et al., 2001) A significant association was also observed between dietary Se and Hsp27 IgG antibody titres ($p < 0.05$).

In the controls, there was a significant positive correlation between total dietary PUFAs and Hsp27 IgM antibody titres ($p < 0.05$) (Table 3). Significant positive correlations were also observed between height and Hsp27, and between age and Hsp27 IgG respectively ($p < 0.05$), and between physical activity and Hsp27 IgM and negatively related to Hsp27 IgG concentrations at ($p < 0.05$ respectively). Age was positively associated with Hsp27 antigen concentrations and Hsp27 IgG levels ($p < 0.05$, respectively). Dietary fat was positively related to serum sICAM-1 concentrations ($p < 0.05$). No significant correlations were found between dietary micronutrients (Zn, Se, Vitamins A, C, and E) and dietary trace element intake in the healthy controls or adolescent gymnasts (Table 3).

Partial correlation analysis was used to explore the relationship between demographic and dietary factors, and biochemical parameters in the gymnast and control groups separately (Table 4). Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity. In the controls, there was a significant positive, partial correlation between dietary fat and serum sICAM-1 [$r = 0.46$, $p < 0.05$]. A positive, partial correlation was also observed between dietary factors (carbohydrate and zinc) and Hsp27 IgG antibody [$r = 0.60$, $p < 0.05$; $r = 0.69$, $p < 0.05$], and between Hsp27 and PUFAs [$r = 0.74$, $p < 0.05$].

Association between markers of cardiovascular disease, dietary fats and anthropometrics

To take account of potentially confounding variables, we used stepwise multiple regression analysis, where hsCRP, sICAM-1, Hsp27, IgM, and IgG were entered as the dependent variable; physical activity, and dietary factors (SFA, MUFAs, PUFAs, and Chol) and anthropometric (weight, height or BMI) were then entered as independent variables (Table 5). The results showed that physical activity was the most significant predictor of hsCRP, contributing approximately 18.5% of the variation

Table 2. Serum hsCRP, sICAM-1, and Hsp27 antigen and antibody titres in gymnast and control. Values are expressed as mean (±SEM).

Biochemical Variable	Controls (n=19)	Gymnasts (n=25)
sICAM (mg/L)	.23 (.01)	.290 (.015) **††
hsCRP (mg/L)	1.38 (.19)	.49 (.03) ***†††
Hsp27 (ng)	7.00 (1.80)	7.07 (1.94)
Hsp27 IgM antibody titres (nm)	.43 (.06)	.38 (.03)
Hsp27 IgG antibody titres (nm)	.14 (.01)	.13 (.02)

ANOVA significant differences ** $p < 0.01$, *** $p < 0.001$; ANCOVA adjusted for height and weight †† $p < 0.01$; ††† $p < 0.001$, compared to the controls; sICAM, intercellular adhesion molecule-1; hsCRP, C-reactive protein; Hsp27, heat shock protein 27; nm, absorbance at 450nm. (n) represents number of subjects.

Table 3. Pearson's correlation coefficients between demographic data, dietary intake and markers of cardiovascular health in gymnasts and control.

Variables	Gymnasts					Controls				
	ICAM-1	hsCRP	Hsp27	Hsp27 IgG antibody	Hsp27 IgM antibody	ICAM-1	hsCRP	Hsp27	Hsp27 IgG antibody	Hsp27 IgM antibody
Demographic:										
Height (m)	-.378	.175	.367	-.032	.147	-.250	-.076	.577*	.353	-.095
Weight (Kg)	-.373	.231	.219	-.086	.210	-.246	.105	.325	.302	-.353
BMI (kg/m^2)	-.119	.250	-.015	-.136	.259	-.113	.382	-.173	.048	-.533
PA (minutes/week)	-.187	.136	-.102	-.166	.166	-.080	-.105	.045	-.430*	.417*
Age (y)	-.285	.083	.338	-.132	.041	-.196	-.149	.599*	.517*	-.229
Dietary factors:										
Total energy intake	-.456	.109	-.350	.277	.126	.287	-.096	.325	.176	-.103
CHO	-.303	.048	-.449	.208	.113	.155	-.093	.356	.200	-.137
Fat	-.534*	.187	-.310	.293	.193	.456	-.110	.094	-.087	.076
SFA	-.549*	.089	-.530	.147	.006	.373	-.144	-.362	.186	-.156
MUFAs	-.487*	.226	-.254	.287	.225	.378	-.236	-.027	.091	.125
PUFAs	-.398	.342	-.101	.367	.366	.136	.117	.224	-.397	.491*
Chol	-.355	.005	.156	.332	-.142	.001	-.248	.062	.064	.217
Dietary micronutrients:										
Zn	-.376	-.278	.245	.143	-.215	.200	.233	.183	.412	-.228
Se	-.240	.032	.275	.494*	.284	.266	-.034	-.214	-.255	.261
Vit C	-.085	.045	.096	-.192	-.064	.001	.028	.110	.046	.102
Vit A	-.378	.280	-.480	.008	.198	.079	.297	.053	.086	-.056
Vit E	-.288	.132	-.441	-.062	.278	.158	-.294	.098	-.013	-.049

BMI, body mass index; PA, physical activity; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Chol, cholesterol; CHO, carbohydrate; sICAM, intercellular adhesion molecule-1; Hsp27, heat shock protein 27; Se, selenium; Zn, zinc; Vit, vitamins; Significant correlation * $p < 0.05$.

($p < 0.002$). Weight explained 20.5% of the variation in sICAM-1 concentration ($p < 0.001$). Dietary SFA explained 14.8% of the variation in Hsp27, and 21.3% of the variation in serum Hsp27 was explained by SFA with Height ($p < 0.03$; $p < 0.001$ respectively). Dietary PUFA intake explained 11.1% of the variation in Hsp27 IgM ($p < 0.02$).

When we entered BMI into the equation (Table 5), the results showed that body mass index was also the most significant predictor of hsCRP, contributing approximately 19.1% of the variation ($p < 0.002$), 29% of the variation in serum hsCRP could be explained by both

Table 4. Partial correlation coefficients between demographic data, dietary intake and markers of cardiovascular health in gymnasts and control.

Variables	Gymnasts					Controls				
	ICAM-1	hsCRP	Hsp27	Hsp27 IgG antibody	Hsp27 IgM antibody	ICAM-1	hsCRP	Hsp27	Hsp27 IgG antibody	Hsp27 IgM antibody
Demographic:										
BMI (kg/m^2)	-.071	.159	.187	-.288	-.009	-.059	.303	.211	-.066	.028
PA (minutes/week)	-.309	.310	-.013	-.602	-.174	-.030	-.076	.011	-.211	-.066
Age (y)	-.440	.347	.464	.065	.282	.078	-.191	.202	.468	-.329
Dietary factors:										
Total energy intake	-.036	-.548	-.620	.358	.147	.143	-.104	.355	.591	.230
CHO	-.201	-.399	-.595	.159	.236	.490	-.071	.438	.605*	.123
Fat	.157	-.635	-.604	.481	.039	.465*	-.107	.040	.004	.338
SFA	-.188	-.297	-.285	.382	-.144	.408	-.172	-.490	.096	.285
MUFAs	-.155	-.429	-.356	.201	-.163	.105	.105	-.095	.204	.362
PUFAs	-.107	-.525	-.332	.179	-.239	.052	-.108	.744*	-.192	.489
Chol	.333	-.504	-.296	.545	-.249	.143	-.104	-.032	.346	.409
Dietary micronutrients:										
Zn	-.283	-.105	-.098	.364	.321	.334	.131	.175	.698*	-.287
Se	-.512	-.251	-.170	.104	.063	.332	-.103	-.701	-.173	.122
Vit C	-.611	.372	.182	-.576	.503	.011	-.095	-.096	-.165	.602
Vit A	-.758	.089	.055	-.398	.343	.101	.305	-.140	.111	-.146
Vit E	-.499	.195	.186	-.177	.293	.215	-.146	.044	-.041	-.161

BMI, body mass index; PA, physical activity; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Chol, cholesterol; CHO, carbohydrate; sICAM, intercellular adhesion molecule-1; Hsp27, heat shock protein 27; Se, selenium; Zn, zinc; Vit, vitamins; Significant correlation * $p < 0.05$.

of BMI with PA ($p < 0.001$), and PA accounted for 10.2% of the variation in sICAM-1 ($p < 0.02$).

Table 5. Association between marker of cardiovascular disease (hsCRP, ICAM-1, Hsp27, and IgM antibody titres) and, physical activity, BMI, weight, height, dietary fats in gymnast and control combined

Variables	R^2	P value
Serum hsCRP		
PA (minutes/week)	.185	.002
BMI(Kg/m^2)	.191	.002
BMI, PA (minutes/week)	.290	.001
Serum ICAM-1		
PA (minutes/week)	.102	.02
Weight (Kg)	.205	.001
Serum Hsp27		
SFA	.148	.03
SFA, Height (m)	.213	.001
SFA, PUFA	.482	.001
Serum Hsp27 IgM titres		
PUFAs	.111	.02

Independent variable entered into the regression equation as variables SFA, MUFA, PUFA, PA, BMI, weight, and height; (ANOVA). PA, physical activity; BMI, body mass index: SFA, saturated fatty acid; PUFAs, polyunsaturated fatty acid.

Discussion

We have investigated the effect of intensive training (>15 hours of gymnastic training per week for competitive events) on markers of inflammation (hsCRP), endothelial activation (sICAM-1) and auto-immunity (Hsp27 antigen and antibody concentrations). The use of young female gymnasts and an aged matched group of normally active girls meant that the likelihood of confounding effects of co-existing cardiovascular disease was minimized. However it is known that there are a number of other potential confounding factors that affect the biochemical parameters that we measured, including adiposity and dietary intake, and we attempted to correct for these in our analysis. Few previous studies have assessed dietary intake in the detail that we have, and no previous studies have investigated the impact of physical activity on serum Hsp27 and its antibody titres.

Effects of physical activity on hsCRP concentrations

Serum hsCRP concentrations have previously been associated with an increased morbidity and mortality from cardiovascular disease (Koenig et al., 1999) and when compared with several other markers of vascular risk, hsCRP was found to be the strongest independent predictor of CV risk (Ridker et al., 2001) and of other conditions predisposing to CVD including diabetes mellitus and altered autonomic tone (Freeman et al., 2002). Serum CRP concentrations are positively associated with weight and fasting glucose, and inversely related to HDL cholesterol concentrations (Piestrzeniewicz et al., 2007). We have found that serum hsCRP concentrations were significantly lower in the young female gymnasts compared to the sedentary females and this remained after correction for anthropometric measures. Our previous results have shown that chronic physical activity causes induction of protective molecules, such as pro-oxidants (Al-Shammari et al., 2010). These protective molecules (trace elements) form part of the anti-oxidant defenses through the up-regulation of their enzymes. The decrease in hsCRP may well be due to the low adiposity in gymnasts and this could offset the large effect due to the physical activity. This may be linked to the reduction in inflammatory cytokine production in fats and muscle and also due to an increase in insulin sensitivity, thereby improving endothelial function and hence reducing body fat. Proinflammatory cytokines and oxidized LDL activate the endothelium and induce the expression of adhesion molecules that are crucial to the recruitment of inflammation cells to the vessel wall. Adhesion molecules are consider to be a markers of endothelial cell activation and inflammation (Frenette and Wagner, 1996). Oxidized LDL may up regulate the expression of adhesion molecules by endothelial cell, elevating serum sICAM-1 (Hulthe and Fagerberg, 2002). The reduction in inflammation may be also related to attenuation of oxidative stress, as fruits and vegetable have been demonstrated to possess anti-inflammatory activity, where the body fat is a source of cytokines inflammation (Middleton, 1998). Cross sectional studies have reported that physical activity is negatively associated with hsCRP concentrations (Geffken et al., 2001) and that the degree of the effect varies with ethnicity (Majka et al., 2009). Intervention studies in adults have reported that physical activity can reduce serum hsCRP levels in individuals with and without cardiovascular disease (Merrill et al., 2008).

There have been a number of studies that have attempted to assess the relationship between hsCRP and dietary factors. Hickling and colleagues have reported that after controlling for BMI, CRP concentrations are significantly related to dietary factors (Hickling et al., 2008) including PUFA intake. Among healthy individuals hsCRP was reported to be inversely related to dietary PUFA (DHA and EPA) (Pischon et al., 2003), although intervention studies have been less clear cut (Madsen et al., 2003).

Effects of physical activity on serum soluble ICAM-1

Intercellular adhesion molecule-1 (ICAM-1) that plays a key role in leukocyte migration and activation is up-regulated on activated endothelial cells. Serum concentrations of its soluble form sICAM-1 have been reported to be increased in athletes after exercise, whereas its expression on leucocytes was correspondingly reduced, affecting their ability to attach to endothelium (Monchanin et al., 2007). Although long duration exercise was found to be associated with an increase serum ICAM-1, the effects of acute exercise were less evident in healthy men (Jilma et al., 1997). We found that serum sICAM-1 concentrations were higher in the gymnasts compared to the control group, and on multivariate analysis sICAM-1 levels were related to levels of physical activity. Furthermore, it has been reported that a high fat meal can cause a significant increase in plasma concentrations of adhesion molecules, including ICAM-1 that was abrogated by ingestion of vitamins C and E (Nappo et al., 2002). On univariate analysis, serum sICAM-1 was positively associated with dietary fat in the gymnasts, just failing to reach statistical significance in the controls (Al-Shammari et al., 2010). Wegge and his colleagues have previously reported that

dietary fat restriction, high fiber and daily physical activity training caused a significant reductions in body weight, BMI, CRP and serum ICAM-1 levels in postmenopausal women at risk of CAD (Wegge et al., 2004).

Relationship between Hsp27 antigen and antibodies and diet and physical activity

Hsp27 is a molecular chaperone whose expression is induced by a number of environmental stressors including oxidative stress (Ferns et al., 2006). Its release may stimulate an autoimmune response, and we have previously reported that Hsp27 antibody titres are altered in patients with established CVD, or CVD risk factors (Ghayour-Mobarhan et al., 2008). In this current study we did not find that Hsp27 antigen and antibody titres differed between the groups of gymnasts and controls, although antigen concentrations were related to dietary saturated fat intake, and dietary PUFA were determinants of Hsp27 antigen concentrations and IgM antibody titres in the study population as a whole. The explanation for why there was no significant association between serum Hsp27 and physical activity may be related to the induction of antioxidant protective mechanisms associated with intensive exercise.

The results of the correlation analysis (Tables 3 and 4) were inconsistent between the two groups. Significant negative correlations were observed between ICAM-1 and macronutrient intake in the gymnasts but not the controls. Concomitantly, ICAM-1 and the Hsp27 antigen concentrations were positively correlated with macronutrient intake and dietary zinc intake. The reasons for these varying associations are unclear and certainly warrant further investigations. We have previously reported altered antioxidant and trace-element status in adolescent female gymnasts, with serum GPx concentrations being significantly higher in gymnasts and SOD concentrations, significantly lower. Interestingly, serum selenium concentrations were also higher in the active females (Al-Shammari et al., 2010).

Blood sample collection and study limitations

The blood samples were collected from non-fasted recruits and there were several limitations of our study. The collection of a fasted blood sample was our only option because the recruitment of subjects was through gymnastic groups who only met as a group in the afternoon/evening, it was not possible to collect the samples in this way. Furthermore, we were not able to collect information on the timing of the menstrual cycle in post-pubertal subjects. Estrogen and progresterone may have affected ROS and hence the anti-oxidant status, for which there is limited information in the literature. There were differences in terms of blood collection between the two groups as stated in the method section of blood collection.

Conclusion

Early clinical and epidemiologic studies established a link between dietary saturated fats, inflammation and physical activity that lead to increases in oxidative stress. Many of degenerative diseases have been affected in terms of inflammation markers and cytokines. Therefore, these markers hsCRP, sICAM-1, Hsp27 antigen and antibodies are part of the processes. Our findings show that young females have an altered profile of inflammatory markers and endothelial activation compared to their less physical active peers and is an area that certainly warrants further attention.

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Key points

- Results showed that adolescent young female gymnasts have an altered serum inflammatory markers and endothelial activation, compared to their less physically active peers.
- Physical activities improved immune system.
- Differences in these biochemical data kept significant after adjustment for body weight and height.

AUTHORS BIOGRAPHY

Eyad ALSHAMMARI
Employment
Faculty of Health and Medical Sciences, University of Surrey. Guildford, UK
Degree
PhD
Research interests
Nutrition, physical activity, inflammatory markers and Bone disease.
E-mail: e.al-shammari@surrey.ac.uk

Shahida SHAFI
Employment
Faculty of Health and Medical Sciences, University of Surrey. Guildford, UK
Degree
PhD
Research interests
Atherosclerosis, CVD, HSP, Growth factors and *in vivo* models of CVD.
E-mail: s.shafi@surrey.ac.uk

Jaana NURMI-LAWTON
Employment
Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK.
Degree
PhD
Research interests
Nutrition, Physical activity, and Bone health.

Dayangku Fatiha Pengiran BURUT
Employment
Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK.
Degree
BSc
Research interests
HSP27, Immune responses and atherosclerosis.
E-mail: D.Pengiranburut@surrey.ac.uk

Susan LANHAM-NEW
Employment
Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK.
Degree
PhD
Research interests
Nutrition and bone health.
E-mail: S.Lanham-New@surrey.ac.uk

Gordon FERNS
Employment
Postgraduate Medical School, Daphne, Jackson Road, Guildford, UK
Degree
DSc MD, FRCPath FRCP
Research interests
Basic cellular mechanisms of atherosclerosis.
E-mail: g.Ferns@surrey.ac.uk

✉ **Professor Gordon Ferns DSc MD, FRCPath**
Director, Institute for Science &Technology in Medicine, Faculty of Health, University of Keele, Guy Hilton Research Centre Thornburrow Drive, Stoke on Trent, Staffordshire ST4 7QB, UK